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THE EFFECT OF X-RAY ON MORTALITY, WEIGHT, LENGTH, AND COUNTS OF ERYTHROCYTES AND HEMATOPOIETIC CELLS IN FINGERLING CHINOOK SALMON, *ONCORHYNCHUS TSCHAWYTSCHA* WALBAUM*

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(Received for publication Jan 6, 1948)

The experiment was devised to test the effect of X-rays upon young rapidly growing salmon of fingerling size, exposed to radiation under laboratory conditions, and is not concerned with radioactive fish actually being in certain rivers. Results of similar experiments upon the eggs and larvae of the same species have been reported upon by Welander (1945). The effect of irradiating adults will appear at a later time.

On January 25, 1944, 13,000 eggs in the eyed condition were obtained from the State of Washington fish hatchery at Soos Creek near Auburn and cared for in the experimental hatchery at the University of Washington. The eggs started hatching February 12 and were hatching rapidly by February 17. The larvae started feeding April 6 and by May 22 had grown to fingerling size of average total length slightly greater than two inches.

The fish were not fed for from 12 to 24 hours before treatment so that their stomachs would be empty. From May 22 to 25 eight groups each consisting of 600 fish were exposed to X-ray receiving single dose treatments of 100 r, 250 r, 500 r, 750 r, 1000 r, 1250 r, 2500 r, and 5000 r. Each group was subdivided into three batches in order to shorten the time of confinement in the exposure chamber. The exposure chamber consisted of a ¾-inch board with an 8 ¾-inch circular hole covered on either side by tightly stretched plastic fly-

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screen and was barely immersed in a large tank so that the top of the chamber was flush with the water level. Water was sprayed into the top of the chamber from several directions for aeration. The temperature of the water was 58 degrees Fahrenheit except for the 5000 r group for which it was 57. The exposure chamber was placed directly under the X-ray tube at a distance of 30 inches from the target, and the rays passed first through a 0.25-millimeter copper filter and then through the intensimeter, which according to information from the X-ray company made, with the intensimeter, a total of 0.5 millimeter of copper and 1 millimeter of aluminum. The machine was operated at 200 kilovolts and 20 milliamperes, so that on the low setting the intensimeter read between 16.5 and 17.0 and the fish received 23.7 r per minute. Dates and durations of exposures were:

Group	100r	250r	500r	750r	1000r	1250r	2500r	5000r
Date in 1944	5/22	5/22	5/22	5/23	5/23	5/23	5/24	5/25
Minutes exposed	4.22	10.53	21.1	31.6	42.2	52.7	105.3	211

Difficulties with the X-ray machine necessitated a 45-minute extension of the exposure period for the first batch of the 5000 r group. A ninth group, the control, designated 0 r, also consisting of 600 fish was neither irradiated nor confined to the chamber. Only after the 5000 r treatment did the fish appear definitely weakened when returned to the holding tank. Several sank to the bottom of the tank, lying either on their sides or backs. The equilibrium of most was disturbed. They seemed partially paralyzed and swam weakly with the long axis of the body out of the horizontal plane. It was common for the fish immediately upon being returned to the tank to rise to the surface and gulp air. After two hours many of the fish seemed to be recovering and showed a quick reaction to jarring of the tank. On the morning of May 26 one day after treatment 54 fish of the 5000 r group were dead on the bottom of the tank. By the morning of May 29 the fish had lost their sluggishness and were feeding almost as well as the other groups.

To test the effect of retention 200 fish were confined to the irradiation chamber for 3.5 hours but were not irradiated. When they were returned to the holding tank they reacted as had those in the 5000 r group except that there were no signs of respiratory difficulties. The next morning seven were dead. A second test was considered desirable in order to ascertain if the method of aerating the water in

the exposure chamber were responsible for the mortality. Two hundred other fish were placed in the exposure chamber and held for 3.5 hours but, instead of spraying water into the chamber from all directions and thus creating agitation and bubbling of air, the water was allowed to flow from the end of a 1-inch hose into the chamber from one side only. The fish thereby received a relatively large volume of water with very little bubbling of air. When returned to the holding tank the fish exhibited the stiffened or partially paralyzed appearance that had been observed when the water was sprayed, and on the following morning eight fish were dead on the bottom of the tank. It appeared that the method of spraying water into the exposure chamber from all sides was not responsible for the initial mortality since as many died when water flowed in gently as when it was sprayed forcibly.

The groups were observed and cared for in 9 identical 80-gallon tanks with plate glass fronts. Water entered as a forceful jet nearly normal to the surface. The outlet of each tank was an open removable pipe extending from bottom to surface, screened at the upper end to prevent loss of fish. After some fish had been lost by jumping out. screens were laid over the tops of the tanks. Daily routine in the care of the fish included removal and recording of dead (Table 1, Column M), siphoning debris off the bottom, and feeding several times a day, offering as much at each feeding as was taken hungrily. The tanks were scrubbed weekly. Samples for histological study were removed periodically (Table 1, Column R). On July 8 the number of fish in each of the surviving 7 groups was reduced to 300 (Table 1) in order to alleviate crowding. On the last day of the experiment standard lengths were taken for approximately 100 fish picked at random from each group.

Calculation of the cumulative percentage mortality was complicated by occasional loss of fish that jumped out, by the periodic removal of samples for histological purposes, and by the reduction of stock on July 8. The removals were compensated for because there would have been a slightly greater number of deaths attributable to treatment had no removals been made. Daily cumulative percentage mortalities of all 9 groups are plotted in Figure 1. Considering the theoretical number of deaths (4.145) in the control group at the end of the experiment to be the expected value, and using the chi-square

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Table 1. Removals (R) and mortalities (M) of nine groups of fingerling chinook salmon.

Group	0-r		100 r		250 r		500 r		750 r		1000 r		1250 r		2500 r		5000 r	
Date	R	M	R	M	R	M	R	M	R	M	R	M	R	M	R	M	R	M
May 22			15		11		16											
23			2		2		2		8		6		6					
24			4		2		4		3		2		2	1	7			
25	1								2		3		2	1	2	1	11	57
26			3		2	1	2								2	1	2	3
27									2		2		2				2	
28															2			
29	3		2		1	1	2									1	1	
30									1		1		2					2
31											1				3			2
June 1						1						1					4	
3						1								1		1		
5	1		1		1	1	1											
6	1								1		1		2		2	1		3
7																1		1
8										1				1		1	2	3
9												1				7		2
10														1		5		4
11														1		3	6*	3
12	3		1		1		1									1		5
13									4		2		2	1		6		8
14															5	5		36
15	4													1		11	7	55
16														1		16		106
17	5											1		1	1	49		106
18																124*		100
19			2		2		2							1		115		46
20									2		2	1	2	1		139		21
21	1											1			6	78		2
22	1													1		4		
23	1													1				
24														1				
26	1		2		2	1	2							2				
27								3	2	1	2		2	1				
28	3						1					1		3				
29												1		1				
30						1												
July 1	1													2				
2			1						1	1		2		2				
3	2		2		2		2	1				1		1				
4					1				2		2		3	2				
6														3				
8	272		265		266		261		269		266		244	1				
10			2		2		2							2				
11	3								3		2		4	1				
13								1						2				
14										1				1				
17			2	1	2		2							2				
18	2								2		2		2					
19								1		1								
20								1						1				
21						1												
24			2		2		2											
25	2								2		2		2					
28														1				
30								1										
31			2		2		2	1										
Aug. 1	2							1	2		2		2	1				
2														1				
3								1				1						
4		1																
7			2		2		2	1		1								
8	2							1	3		2		2					
10														1				
13										1				1				
14			2		2		2	1										
15	2								2		2		2	1				
16								1						1				
17		1				1								1				
18														1				
19										1		1						
Total	313	2	312	1	305	9	308	15	311	6	302	12	283	51	30	570	35	565

* Arbitrary reduction by one fish in the case of the 2500r group, and allocation of six fish in the case of the 5000r group, in order to adjust for discrepancies in totals.

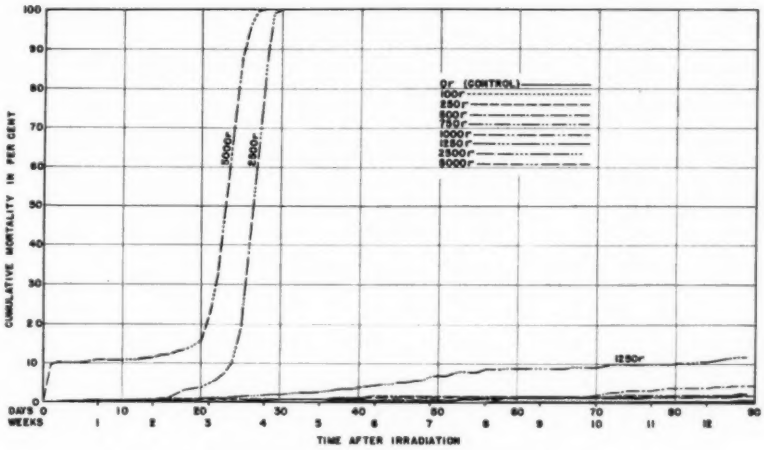


FIGURE 1.
GRAPH SHOWING COMPENSATED DAILY CUMULATIVE PERCENTAGE MORTALITY OF FINGERLING CHINOOK SALMON SUBJECTED TO VARIOUS AMOUNTS OF X-RAY.

test with Yates' "correction for continuity" (Snedecor, 1940: 169), the mortalities among groups irradiated with 250 r or more were shown to be significantly, at the 1 per cent level, greater than would be expected from chance variation, while in the 100 r group the mortality was not significantly different from the control mortality.

Weights of two, or in the case of the 100 r group on August 3, four samples of 100 fish from each of the 9 groups were determined at intervals of two weeks. The weights for each group and date, hereafter called a group-interval, were averaged as the basis for the graphs of Figure 2 which show that growth in weight was in a general way inversely related to amount of X-ray received. At the end of the experiment the 1250 r group weighed only about 70 per cent as much as the controls, and other groups tended to be intermediate. The significance of the difference in rate of growth of each of the first six irradiated groups from the rate of growth of the control was tested by the method of Fisher (1938: 146-148). The regression coefficient of the irradiated group when compared to that of the control showed a significant decrease at or beyond the 1 per cent level of "t" in groups receiving 500 r or more. The growth rates of the 100 r and 250 r groups were not significantly different from the control.

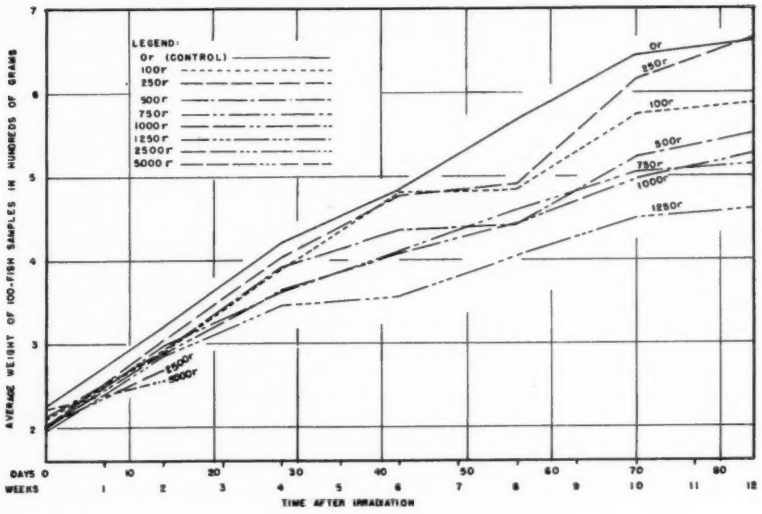


FIGURE 2.
GRAPH SHOWING INCREASE IN WEIGHT WITH TIME AFTER IRRADIATION OF VARIOUS GROUPS OF FINGERLING CHINOOK SALMON.

At the end of the twelfth week on August 17, standard lengths in millimeters to the ends of the silver areas of the bodies were measured individually for approximately 100 fish in each of the surviving 7 groups. The light histographic lines in Figure 3 show the actual lengths while the heavy lines show the lengths smoothed by a moving average of 5 millimeters. Length was significantly affected (reduced) only in the case of the groups receiving 1000 r or more. The 100 r mean differed very little from the control. The mean length of the 250 r group considerably exceeded that of the control, but the difference was not significant partly because of the relatively high standard deviation of the 250 r group. The means of the 500 r and 750 r groups were not significantly shorter than the control. For the 1000 r group the difference was of borderline (1-5 per cent level of "t") significance, but for the 1250 r group it was significant, both groups being shorter than the control.

Cell counts of the circulating blood of most of the fish later used for histological purposes were made in the fresh condition with the

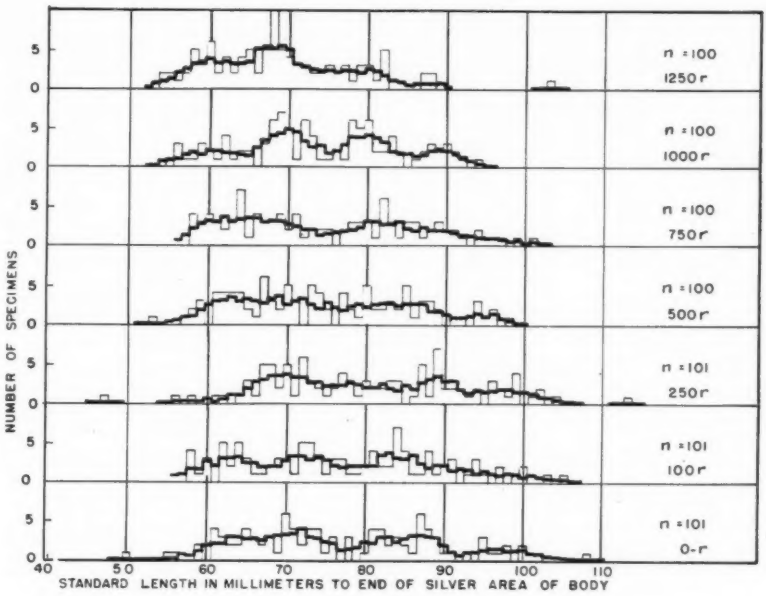


FIGURE 3.
HISTOGRAMS OF STANDARD LENGTHS (LIGHT LINES) AND THE SAME LENGTHS SMOOTHED BY A MOVING AVERAGE OF 5 MILLIMETERS (HEAVY LINES) OF 7 SAMPLES OF FINGERLING CHINOOK SALMON 12 WEEKS AFTER IRRADIATION.

use of diluting pipettes and counting chambers. Methods usually used for counting mammalian red blood cells were employed. Attempts to count white cells in the fish blood were unsuccessful because no method was known for complete lysis of the red blood cells whose nuclei resisted treatment as much as did the white cells. The presence of a preponderance of red cells made counting of the relatively scarce white cells very time consuming. White cells were distinguishable on smears, but their distribution over the surface of the slide was not uniform and the distinction between white and red cells could not be made with enough certainty to justify making white cell counts even though it was expected that white cells as in the case of mammals should be highly sensitive to irradiation. Blood was taken from heart incisions during the early part of the experiment, but a better method

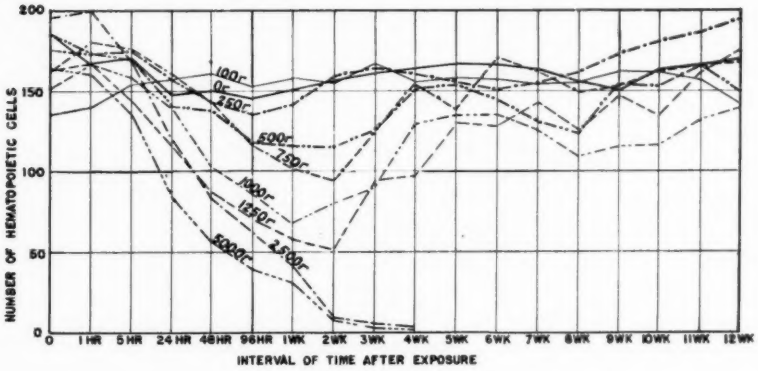


FIGURE 4.

GRAPH SHOWING NUMBERS SMOOTHED BY A MOVING AVERAGE OF 3 INTERVALS OF TIME OF HEMATOPOIETIC CELLS PER 60,000 CUBIC MICRA OF KIDNEY DURING THE FIRST 12 WEEKS AFTER VARIOUS DOSES OF X-RAY.

used throughout the rest of the experiment was amputation at the caudal peduncle and the use of the drop of blood collected at the cut end of the body. In comparison with human blood, the blood of fingerling salmon clots very quickly so that unusable dilutions were obtained unless the dilutions were completed within about 5 seconds of the time of the incision. Too low a count caused by clotting in the capillary tube of the pipette was the usual result of delay in getting the sample. When a clot was observed in the pipette the count was not used. Early in the experiment ordinary blood pipettes were used, but later the small "fish" pipettes with a total capacity of about 0.3 cubic centimeters were used to supplement the use of ordinary pipettes, half of the counts being made with either type. During the last two weeks a standard sampling practice was followed of making four counts for each of two fish. A total of 729 counts not evenly distributed among 240 fish were made. Comparing the mean count of circulating blood cells for each group-interval (table 2) with the mean of all 729 counts gave values of "t" at or beyond 1 per cent for scattered high means mostly during the later weeks of the experiment. Except for one isolated low mean for the 750 r group at 0 time, the low means occurred in the higher irradiations from 1 to 4 weeks after exposure. The high means reflecting the general rising

TABLE 2.
Mean of blood cell counts regardless of fish averages, for each group-interval with those values indicated which differ from the mean of all 729 counts by an amount sufficient to yield a "t" value at or beyond the 5 per cent level.

Interval	Group								
	0r	100r	250r	500r	750r	1000r	1250r	2500r	5000r
0	99*	102	113	97*	78**	124	129	116	125
1 hour	128	112	114	132		98	144	101	112
5 hours	152	82*	86*	105	118	178	131	107	106
24 hours	153	137	123	174*	134	150	158*	122	124
48 hours	174*	156	111	121	104	90	126	126	110
96 hours	156	138	137	124	166*	108	132	114	116
Average first 96 hours	138	125	114	123	120	128	138	113	118
1 week	141	122	118	132	130	110	112	140	82**
2 weeks	160	146	126	138	126	116	105*	126	103**
3 weeks	120	118	125	116	102**	109	100*	114	92**
4 weeks	116	131	142	114	112	112	112	76**	
5 weeks	150*	125	131	117	127	124	110		
6 weeks	134	150*	146*	145*	134	119	126		
7 weeks	150	140	144	161**	130	126	109		
8 weeks	136	146	139	116	116	172**	132		
9 weeks	144	140	139	134	137	147*	140		
10 weeks	134	143	139	123	130	146*	130		
11 weeks	138	123	153**	126	122	174**	139		
12 weeks	143	120	156**	123	173**	138	109*		

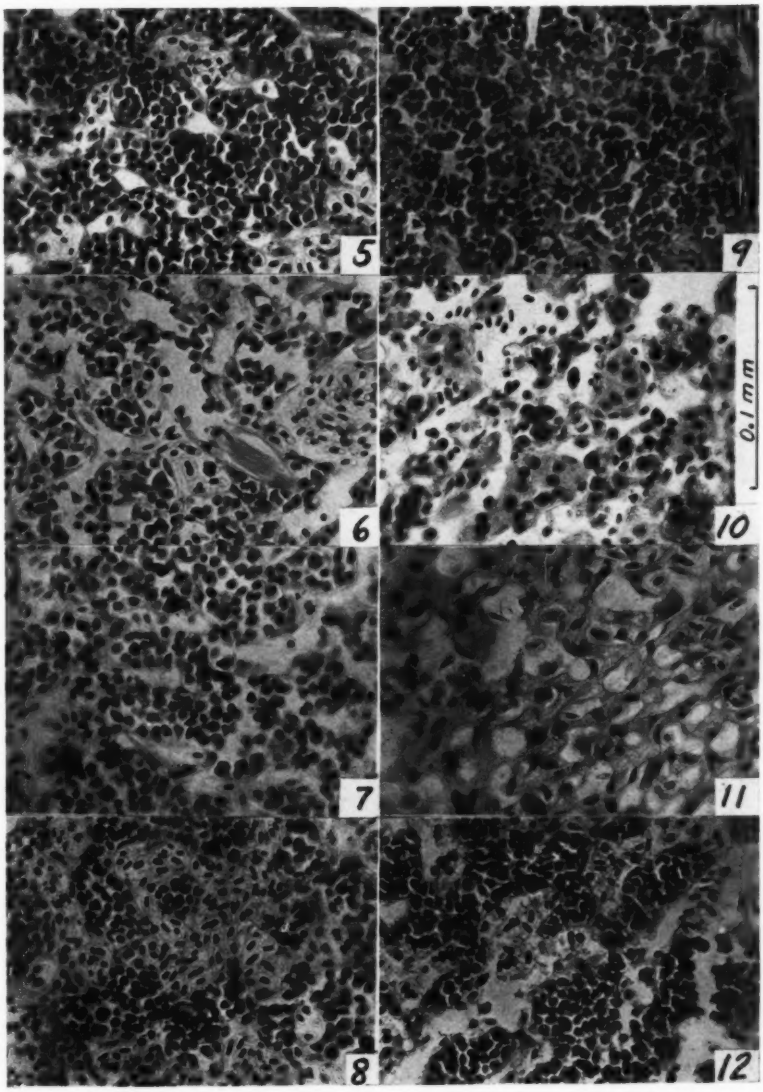
*Value of "t" from 1 to 5 per cent level.
**Value of "t" at or beyond 1 per cent level.

trend toward the end of the experiment are unexplained. The low mean of the 750 r group at 0 time was based upon only 2 counts and may probably be disregarded. The means are low at 2, 3, and 4 weeks in groups receiving more than 750 r, and it is felt that the small number (2-6) of counts in each of these group-intervals accounts for some of the groups not being significantly low.

It is concluded from the blood counting that the average concentration of about 1,270,000 cells per cubic millimeter in the circulating blood of fingerling chinook salmon dropped noticeably in heavily irradiated groups from 1 to 4 weeks after irradiation with doses of from 1250 r to 5000 r, reaching a minimum of between 750,000 and 1,000,000 cells per cubic millimeter just before the death of those given lethal X-ray doses. It is to be noted that decreased blood cell count does not appear so early or in such marked degree as another criterion of X-ray damage, decreased hematopoietic cell count in the kidney, to be discussed next.

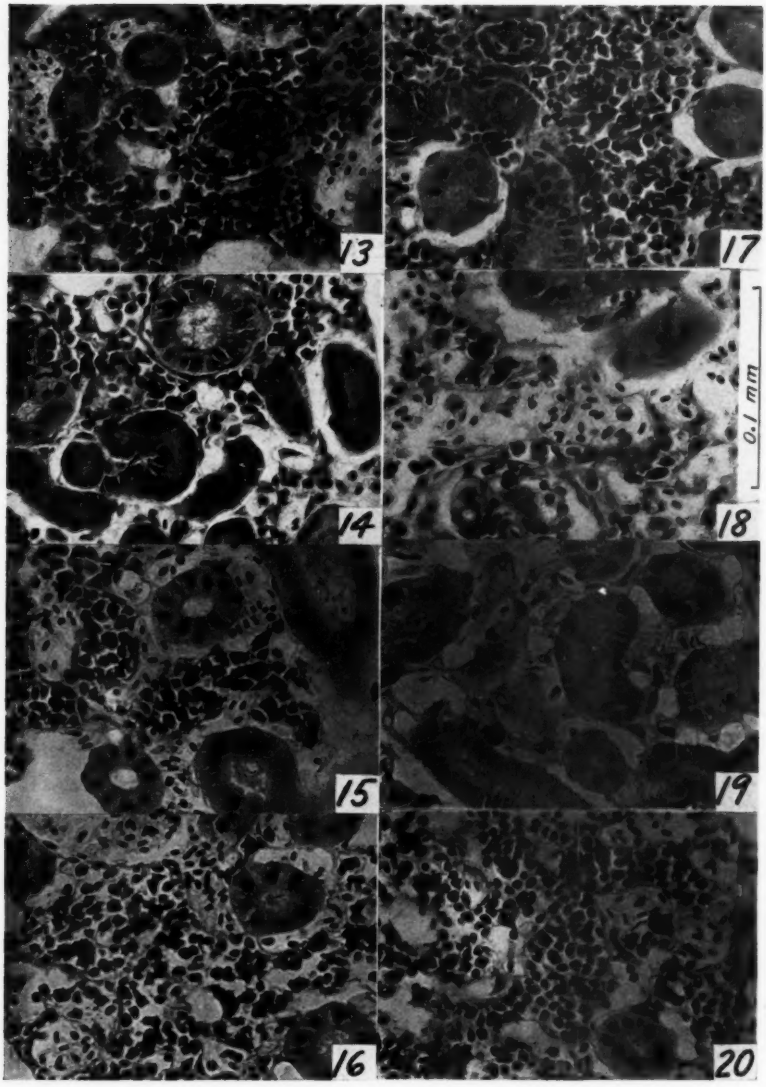
Samples of one or more fish were taken at random from each of the 9 groups at intervals after irradiation as follows: immediately (actually from 2 to 30 minutes), 1 hour (actually from 1 to 1¾ hours), 5 hours, 24 hours, 48 hours, 96 hours, 1 week, 2 weeks, 3 weeks, and 4 weeks (actually from 3½ to 4 weeks). By the end of the fourth week all of the fish in the 2500 r and 5000 r groups had died, so that only the remaining 7 groups were sampled 5, 6, 7, 8, 9, 10, 11, and 12 weeks after irradiation when the experiment was terminated. Including controls 146 fish, each representing a different group-interval, were used for the counts. Total lengths to the tips of the shortest middle caudal rays averaged 64 with a standard error of 0.76 millimeters, and ranged between 47 and 84 millimeters. Actual measurements in most cases were made to the anterior edge of the insertion of the adipose fin and were converted to total length, for those specimens whose tails had been cut off for blood samples. The body cavities of about half of the specimens had been opened before preservation. Preservation was with Bouin's picro-formol-acetic solution which was replaced after 24 hours with 70 per cent ethyl alcohol, renewed at least once. From each sample of one or more fish preserved a single specimen was used to represent each group-interval. In order to facilitate comparison and counting, all samples subjected to the graded series of X-ray doses and the control for one time interval were embedded and sectioned together. Slight differences in section thickness and staining were thus minimized. The anterior kidney extends ventrolaterally as wing-like projections making this portion wider than the rest. As may be seen from Figures 5-12 the anterior kidney consists largely of hematopoietic tissue without tubules or glomeruli. In the rest of the kidney tubules predominate (Figures 13-20). The anterior kidney was noticeably thin and transparent when dissected from the 2-4 week samples of the 2500 r and 5000 r groups.

Counting of cells was done with a binocular monobjective microscope, one eyepiece of which was fitted with a grid consisting of 100 squares arranged in 10 rows of 10. Selection of areas where hematopoietic cells were most closely compacted was decided upon as being the method most likely to bring out the effects of irradiation and at the same time keep experimental error in the counts at a minimum. In selecting areas, artifacts were avoided, and the grid squares that were to be used were so oriented that the maximum number of hema-



FIGURES 5-12 INCLUSIVE.
PHOTOMICROGRAPHS OF ANTERIOR KIDNEY.

FIGURE 5. CONTROL AT 1 HOUR.
FIGURE 6. 1000 R AT 48 HOURS.
FIGURE 7. 1000 R AT 4 WEEKS.
FIGURE 8. 1000 R AT 12 WEEKS.
FIGURE 9. 5000 R AT 1 HOUR.
FIGURE 10. 5000 R AT 48 HOURS.
FIGURE 11. 5000 R AT 4 WEEKS.
FIGURE 12. CONTROL AT 12 WEEKS.



FIGURES 13-20 INCLUSIVE.
PHOTOMICROGRAPHS OF MIDDLE KIDNEY.

FIGURE 13. CONTROL AT 1 HOUR.
FIGURE 14. 1000 R AT 48 HOURS.
FIGURE 15. 1000 R AT 4 WEEKS.
FIGURE 16. 1000 R AT 12 WEEKS.
FIGURE 17. 5000 R AT 1 HOUR.
FIGURE 18. 5000 R AT 48 HOURS.
FIGURE 19. 5000 R AT 4 WEEKS.
FIGURE 20. CONTROL AT 12 WEEKS.

topoietic cells fell within the counting area. When the area of greatest concentration of hematopoietic cells had been selected, all of the nuclei within the area were enumerated. Those cells were identified as hematopoietic which had dark compact nuclei and which were not identifiable as some other type. What was estimated to be at least one-half of a nucleus was tallied as a whole nucleus if a cell were split. In cases of severe damage from 1 to 4 weeks after intense irradiation (Figures 11 and 19) normal hematopoietic cells were lacking, but the remaining pale or otherwise abnormal-appearing nuclei that could not be definitely assigned to other categories were considered as hematopoietic. In the areas chosen for counting, hematopoietic cells constituted 77 per cent of the total. A measure of the variability in the counts was obtained by counting 24 fields involving 3 control fish, all preserved at approximately the same time. The mean of the 24 counts was 78.7 and the standard deviation was 13.0. The counts of hematopoietic cells in anterior and middle kidney showed similar trends, and were added to give the total count for the kidney (Table 3). Figure 4 shows graphically the data of Table 3 smoothed by a

TABLE 3.

Hematopoietic cell count in 12,000 square micra of 5-micron sections of kidney, summing corresponding counts of anterior and middle kidney, and designating with asterisks those sums which differ significantly from the mean (135.9) of the 146 control counts.

Interval	Group								
	0r	100r	250r	500r	750r	1000r	1250r	2500r	5000r
0	180	145	151	166	169	148	162	178	142
1 hour	194*	125	203*	205*	161	158	164	216*	187
5 hours	130	151	165	149	214*	199*	175	207*	150
24 hours	185	183	159	160	146	120	89	77*	68*
48 hours	130	138	151	110	123	98	81*	70*	30*
96 hours	139	158	120	144	161	89	92	100	72*
1 week	169	159	136	100	67*	74*	36*	16*	16*
2 weeks	145	157	(169)	103	74*	41*	45*	9*	3**
3 weeks	158	145	175	143	138	124	75*	3**	4**
4 weeks	180	172	152	132	160	104	74*	6**	3**
5 weeks	153	146	155	178	166	159	143		
6 weeks	172	156	161	153	191*	143	174		
7 weeks	175	169	138	103	156	103	68*		
8 weeks	144	135	168	136	139	127	186		
9 weeks	149	159	179	126	154	96	123		
10 weeks	159	189	172	199*	166	119	133		
11 weeks	179	135	193*	135	166	133	145		
12 weeks	162	147	195*	163	170	142	204*		

*Value of "t" between 1 and 5 per cent.
**Value of "t" at or beyond 1 per cent.

moving average of 3 to remove excessive irregularities caused by individual variation. There was a decrease in number of hematopoietic cells in the kidneys of specimens receiving 500 r or more, starting between 5 and 24 hours, dropping to a minimum in the second week, and leading to death by the fourth week in those fish irradiated with 2500 r and 5000 r, or leading to recovery or near recovery about the fourth or fifth week in the case of the survivors in groups irradiated with from 500 r to 1250 r. The three groups least affected (0 r, 100 r, and 250 r) maintained fairly uniform numbers per unit area. Values differing significantly as determined by the "t" test from the mean of all 146 counts in Table 3 are indicated by asterisks. Fiducial limits at the 5 and 1 per cent levels of "t" were respectively 82.2-189.7 and 8.1-263.7. Significantly low counts at or beyond the 5 per cent level of "t" in Table 3 are arranged roughly in the form of a triangle with base to the right from 24 hours to 4 weeks and apex toward the left at the 1 and 2 week 750 r group-intervals. Significantly high counts are scattered and are not explained. The only values at or beyond the 1 per cent level were the low counts at 3 and 4 weeks in the 2500 r group and at 2, 3 and 4 weeks in the 5000 r group. The hematopoietic tissue of the kidney of fingerling chinook salmon is concluded to be highly susceptible to damage by X-rays as is indicated by the progressive decline of the cell count with increased irradiation. Doses of 500 r and more produced a detectable effect within 24 hours and the greatest decrease in abundance occurred between 1 and 2 weeks after irradiation.

When these results of irradiating fingerling chinook salmon are compared with those of Welander (1945) on the larvae it is seen that in the groups subjected to higher irradiations (2500 r or more) the larvae survived slightly longer than did the fingerlings, but that these doses were ultimately lethal for both stages. Comparing the lowest X-ray doses producing significant effects in the fingerlings with the doses affecting the larvae we find similarity:

Characteristic	Larvae		Fingerling	
	Dose in r	Time after irradiation of greatest effectiveness, weeks	Dose in r	Time after irradiation of greatest effectiveness, weeks
Mortality (50 LD)	1000	14	1250-2500	4+
Length increase	500	1 - 18	1000	12—
Weight increase	1000	10 - 18	500	12—
Hematopoietic cell count of kidney	250	4 - 5	500	2 - 3

Among the larvae, diminished hematopoietic cell counts occurring in the 250 r and 500 r groups were most extreme after from 3 to 6 weeks, while the 1000 r group remained low at least until the tenth week. The number of hematopoietic cells in the kidney of the control group of larvae increased during this 13-week period in the life of the fish.

Summary

1. Eight groups of fingerling chinook salmon, *Oncorhynchus tschawytscha* Walbaum, were subjected to different X-ray doses graduated from 100 r to 5000 r, and one group was used as a control.
2. The lowest exposure levels at which irradiated groups were significantly adversely affected 12 weeks after exposure were, in mortality 250 r, in weight 500 r, and in length 1000 r.
3. Group means when tested against the mean of all 9 groups combined showed significant decline in number of cells in the circulating blood at exposures of 750 r and 1250 r 2 and 3 weeks after irradiation.
4. Concentration of hematopoietic cells in the kidney showed similar decline in the 750 r group 1 and 2 weeks after irradiation.

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THE PRENATAL GROWTH OF THE CAT. XVI. CHANGES IN THE RELATIVE WEIGHTS OF THE ORGANS

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It has been known for a long time that the rate of growth of the body is not constant throughout the growth period and also that the various organs and parts have different growth rates. It is also known that these individual organ rates of growth change during the growth period. In the plan of his contemplated book, Leonardo da Vinci says, "Then you will describe what members are those that grow more than the others after the child's birth" (McMurrich, 1930). In 1759, de Montbeillard began a study of the growth in the weight and in the stature of his newborn son and this growth curve shows fluctuations in the postnatal growth of this boy from birth to 18 years of age, (Scammon, 1927). Meyer (1914), in a study of the prenatal growth of man, speaks of its "periodicity". Bean (1924) describes these wave-like fluctuations in growth as "the pulse of growth." Davenport in 1926 calls these changes in the rates of growth "metamorphosis." He says, "growth of the body is the resultant of several growth-promoting internal stimuli. These act at different times and upon different organs and this is the essence of metamorphosis." These fluctuations of growth are found not only in man but also in most of the higher animals studied thus far. Brody (1945) shows the variations in the general body growth of many animals.

Scammon (1942) has arranged most of the organs of the human body in four general types of growth. Dunn (1921) has shown the variations in the prenatal growth of the various parts of the human brain. There have been many other studies on the growth of the various organs in man and in many of the mammals. Donaldson (1923) has summarized much of the postnatal growth of the albino rat, showing the variations in the growth of the individual organs, both in time and also in their relation to the body weight. The variations in the

rate of growth of the individual organs in the embryonic chick has been studied by Schmalhausen (1927) and he has named this type of growth in which the various organs do not grow at the same rate throughout the embryonic period, "heteromorphe Wachstum". Kaufman (1930) in a study of the prenatal growth of the chicken and the pigeon, finds that the eight organs studied do not have the same growth rate throughout the period studied and also that the rates of each are different from the rates of growth of the other organs. The growth of the same organ in the two birds is not the same. Wallace (1945) shows this same variation between organs and also in the same organ at different periods of prenatal growth for the sheep.

Some organs have their most rapid growth early, and others grow most rapidly in the latter part of the fetal period, and as a result there is an ever-changing proportion of the various organs and parts of the body. The weight of the entire body at any time is really a summation of the weights of the various organs and parts at that particular time. The growth of any organ may be studied separately and its changes in rate of growth studied, but it is really a part of the entire body, affecting, and being affected by, the changes in the other organs. Some organs naturally have a greater effect in modifying the growth of other organs. For example, all of the organs receive blood pumped by the heart and the kidneys excrete the waste material of the other organs. Large organs or systems such as the musculature, in their periods of rapid growth, would naturally modify the percentages of the smaller organs, such as the hypophysis. Thus, there is a continually changing interrelationship between the various organs and parts of a growing animal.

The absolute and percentage growth of the individual organs of the cat have been described in the earlier papers of this series (see references.) This paper will attempt to compare the changes in weight and in percentage weight of the various organs throughout the fetal period, in the newborn kitten and in the adult cat. The only data, so far as is known, on the postnatal growth of any of the organs of the cat is the work of Hall and MacGregor (1937) on the postnatal growth of the kidney. There are no known data on the changes in the other organs in the postnatal period in the cat, and so only the weights and the percentages in the adult are available.

Changes in Weight

The increase in absolute weight given as multiples of the initial weight at the beginning of the fetal period is shown in the first column of Table 1. Seven organs increase more than the total body weight and 14 organs increase less than the total body weight increase. The thymus which has the greatest increase in the fetal period, increases five and one half times as much as the total body weight and the hypophysis increases the least, or but 5.9 per cent of the increase of the total body weight.

The second column of this table gives the increase between birth and maturity. Separate columns are given for the males and females as the weights are different in the two sexes in the adults. There was but a very slight difference during the fetal period and both sexes were grouped together.

The postnatal increase in the body weight of the males is a little

TABLE 1

Increase in the Body Weight and in the Various Organs, Expressed as Multiples of the Weight in Grams at the Beginning of the Fetal Period.

Increase in prenatal period		Increase in postnatal period		
			Males	Females
Thymus	1060.6	Testes	134.4	——
Kidneys	925.8	Musculature	35.6	31.7
Dig. tube wgt.	814.3	Spleen	31.7	23.7
Integument	694.3	Pancreas	27.7	26.7
Spleen	598.0	Skeleton	26.6	22.9
Lungs	360.6	Spinal cord	24.9	23.9
Musculature	287.3	Dig. tube wgt.	24.8	22.8
Body weight	191.1	Body weight	23.5	20.4
Eyeballs	174.0	Uterus	——	19.9
Liver	138.4	Liver	20.9	18.3
Skeleton	125.5	Kidneys	20.7	16.6
Heart	76.2	Suprarenals	15.7	14.5
Brain	63.9	Integument	14.8	12.1
Thyroid	57.3	Ovaries	——	11.8
Ovaries	29.0	Eyeballs	14.1	14.0
Pancreas	28.8	Heart	12.4	10.9
Suprarenals	25.4	Lungs	9.4	8.4
Spinal Cord	24.4	Thyroid	8.2	7.6
Testes	23.7	Brain	7.8	7.5
Dig. tube length	22.1	Hypophysis	6.4	5.9
Uterus	15.2	Dig. tube length	3.5	3.3
Hypophysis	11.2			

over one eighth the increase in the body weight in the fetal period and a little over one ninth for the females, or the females do not increase quite as much in the postnatal period as the males. There are also seven organs or systems in the males which have a greater increase than the total body increase, but of these, only three are common to both the pre- and postnatal periods, namely the musculature, the weight of the digestive tube and the weight of the spleen. The musculature has next to the largest increase in the postnatal period, but its increase in the fetal period is only slightly greater than the body weight. The testes have the greatest postnatal increase, while the ovary is in about the middle of the group of organs which increase less than the body weight in the postnatal period. The ovary does increase a little more during the fetal period than do the testes. May this be due to the stimulus of female hormones during the intrauterine period? The other six organs which increase more than the total body weight are the same in both sexes, but their order is not the same except the musculature which is the greatest of the six in both sexes except for the testes in the males and the weight of the digestive tube which is the lowest in both sexes. Each of these six parts increases more in the male than in the female in the postnatal period.

The liver, the kidneys, and the suprarenals increase a little less than the total body weight in the postnatal period and they are in the same order in both sexes. The six organs with the lowest increase are in the same order in both sexes and they occur in the following order of decreasing ratios to the newborn weight: heart, lungs, thyroid, brain, hypophysis and the length of the digestive tube. This last measurement is a linear measurement and naturally its value might be expected to increase less than the weights of the other organs. In the postnatal growth period, the increase of the uterus has the greatest value, but its increase is next to the lowest in the fetal period. The brain, the eyeballs and the hypophysis increase less than the total body weight in both the pre- and the postnatal periods. The hypophysis has the lowest increase in both pre- and postnatal periods in both sexes, with the exception of the linear increase of the digestive tube. Its ratio, as will be shown later on in Table 4 and in Figure 1, shows a more marked decrease in rate of relative growth than found in any of the other organs in the fetal period. The heart is found about midway in both pre- and postnatal groups. It, like the brain,

has grown very rapidly in the embryonic period, and so their growth is less than the total body weight in both the prenatal and the postnatal periods.

The total increase from the beginning of the fetal growth to maturity is shown separately for the sexes in Table 2. In this table there

TABLE 2
TOTAL INCREASE IN THE BODY WEIGHT AND IN THE VARIOUS ORGANS FROM THE BEGINNING OF THE FETAL PERIOD TO MATURITY

	Males	Females
Dig. tube wgt.	20,177	18,534
Kidneys	19,198	15,355
Spleen	18,925	14,175
Integument	10,298	8,374
Musculature	10,236	9,118
Body Weight	4,493	3,894
Lungs	3,371	3,022
Skeleton	3,339	2,870
Testes	3,126	——
Liver	2,900	2,532
Eyeballs	2,448	2,430
Heart	942	829
Pancreas	797	770
Spinal Cord	608	585
Brain	497	479
Thyroid	470	436
Suprarenals	399	368
Ovaries	——	341
Uterus	——	302
Dig. tube	77	73
Hypophysis	72	66

are but five organs or systems which grow more rapidly than the entire body weight and of these but three are found in the upper divisions of the pre- and postnatal growth (Table 1) and the total growth. They are the weight of the digestive tube, the spleen, and the musculature.

The three parts having the smallest increase in Table 2 are the length of the digestive tube, the weights of the uterus and the hypophysis. These three were also the lowest in Table 1. There are some variations, but on the whole the relative positions of the various organs is much the same in this table and in the preceding table. The increases are larger of course, for this table gives the total increase from beginning of the fetal period to the adult. The total postnatal increase in the

hypophysis, and the brain are very low. The organs growing the least in postnatal life are the parts of the nervous system (with the exception of the spinal cord), the thyroid, lungs and heart, and the one linear dimension, or the length of the digestive tube.

The averages of the increases in the two tables has been determined. It gives the general trends of growth in the fetal period and for the two sexes in the postnatal period. The average increase of the body weight and of all the organs except the testes, ovaries and the uterus is 284.7 times for the fetal period, and 18.3 times for the males in the postnatal period and 16.2 times for the females in the postnatal period. The average increase during the fetal period is 15.5 times that of the male average in postnatal life. The average increase of the males exceeds that of the females in the postnatal period by 12.88 per cent. This again shows that growth when compared to the initial masses is greater in the fetal than in the postnatal period, and also that the increase after birth is greater in the males than in the females.

Tables of total increase in the postnatal period are given for the rat (Donaldson, 1923) and for the chicken (Latimer, 1925). The testes shows the greatest postnatal growth in the cat and also in the rat. The relative growth of the testes is not given for the chicken. The musculature is second in the cat and also next to the greatest in the chicken, but it is not given for the rat. The spleen is high in the cat and rat, and a little below the total increase of the body weight for the chicken. The pancreas is high in both the cat and chicken and the digestive tube weight is high in the cat and rat but lower than total body weight in the chicken. Thus, we see that the organs of movement, of digestion (liver not included, due probably to its early prenatal development and function) and of reproduction in the male grow more than the entire body. The growth of the brain and the hypophysis in the cat, rat and chicken during the postnatal period is very much less than the total body weight increase.

The periods of development in which the organs and systems attain their maximum percentage weight is shown in Table 3. At the beginning of the fetal period the parts of the central nervous system, including the hypophysis are at their maximum percentage of the body weight, also the heart which is likewise generally recognized as precocious in its growth. The gonads, two of the endocrine glands and the skeleton complete the list. The liver attains its maximum shortly

TABLE 3
PERIODS OF ATTAINMENT OF MAXIMUM PERCENTAGE WEIGHT OF THE VARIOUS ORGANS

0.3 grams or beginning of fetal period,	Brain Spinal cord Hypophysis Heart Ovaries Thyroid Suprarenals Testes Skeleton
10 grams	Liver
120 grams or Newborn	Lungs Thymus Kidneys Integument Uterus Pancreas Dig. tube weight Eyeballs
2822 grams, or Adult male	Spleen Musculature Length Dig. tube

after the beginning of the fetal period or at 10 grams of body weight. This may be due to its various functions.

Some of the organs shown as attaining their maximum percentages at birth, or 120 grams of body weight, may attain a greater maximum later on in the postnatal period for which we have no available data. The postnatal percentage changes of the rat, (Donaldson, 1923) and the chicken, (Latimer, 1925) show that several of the organs, especially the digestive tube and its parts, have maxima in the early part of the postnatal period. The spleen, the musculature, and the length of the digestive tube do not attain their maximum percentages until the adult weight is reached, so far as these data show. As has been stated, we have no data on the changes which take place during the postnatal period. We know only the weights in the newborn and then in the adult and it is quite possible that several of these organs may develop in the early part of the postnatal period to a maximum greater than that shown at birth. For example, in the postnatal development of both the rat and the chicken, the parts of the digestive tube develop rapidly in early postnatal life to enable the animal to take care of the

more complex food usually eaten by the adult. This table gives the data without these possible, and in fact probable, postnatal changes.

Changes in Percentage Weight

The increase in weight for the fetal period, for the postnatal period and for the total increase in weight from the beginning of the fetal period to the adult have been given. Then the periods of attainment of the maximum percentages are shown. All of these first three tables show that there is a great diversity in the growth of the several organs. A direct comparison of the various percentages was attempted but it was very difficult to present this graphically for there is such a wide variation in the percentage values. For example, the musculature varies from 20 to 50 per cent of the body weight while the percentage weight of the hypophysis decreases from 0.07 to 0.001 per cent.

To more readily compare the changes in these percentages, the percentage weight in the adult male was taken as unity and the per-

TABLE 4

Ratios of the Percentage Weights to the Adult Male Percentage Taken As Unity. Percentages of Adult Female Were Used for Ovaries. These Data Are Shown Graphically in Figure 1.

Body (grams)	Heart	Liver	Eyeballs	Hypophysis	Thyroid	Brain	Spinal cord	Suprarenals	Ovaries
0.3	5.08	1.39	1.86	51.47	9.05	8.73	7.88	11.57	12.22
10	2.41	2.01	1.54	14.34	6.25	3.98	1.54	5.36	2.78
20	1.93	1.76	1.53	8.31	5.18	3.30	1.03	4.01	1.89
30	1.93	1.57	1.54	6.25	4.41	2.99	1.03	3.36	1.80
40	1.93	1.44	1.57	5.88	4.11	2.77	1.03	2.86	1.80
50	1.93	1.32	1.61	5.52	3.81	2.62	1.03	2.41	1.80
60	1.93	1.22	1.66	5.15	3.69	2.62	1.03	2.07	1.80
70	1.93	1.15	1.68	4.78	3.51	2.76	1.03	1.93	1.80
80	1.93	1.14	1.67	4.56	3.35	2.91	1.03	1.79	1.80
90	1.93	1.14	1.65	4.27	3.21	2.91	1.03	1.79	1.80
100	1.93	1.14	1.61	3.97	3.04	2.91	1.03	1.79	1.80
110	1.93	1.14	1.63	3.68	2.86	2.91	1.03	1.79	1.80
120	1.93	1.11	1.75	3.53	2.74	3.01	1.03	1.79	1.80
130	1.93	1.10	1.97	3.24	2.71	3.20	1.03	1.79	1.80
140	1.93	1.08	2.17	3.09	2.68	3.30	1.03	1.79	1.80
150	1.93	1.07	2.08	2.94	2.63	3.30	1.03	1.79	1.80
160	1.93	1.06	1.89	2.79	2.62	3.30	1.03	1.79	1.80
170	1.93	1.07	1.78	2.50	2.62	3.30	1.03	1.79	1.80
180	1.93	1.10	1.71	2.43	2.62	3.30	1.03	1.79	1.80
190	1.93	1.13	1.68	2.21	2.58	3.30	1.03	1.79	1.80
200	1.93	1.11	1.67	2.06	2.56	3.30	1.03	1.79	1.80
Adult	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

centages at each ten gram intervals of body weight throughout the fetal period, and also for the newborn period, were determined as ratios of the percentage in the adult. These ratios are given in Tables 4, 5 and 6 and these ratios have been plotted in the following four figures to graphically represent the relative changes in each of these viscera from the beginning of the fetal period to birth and then in the adult. The end of the newborn period and the adult are connected in the figures by dotted lines for we have no data for this interval and this dotted line is merely a point to point line, or it merely connects the newborn and the adult.

The percentages at the beginning of the fetal period were taken as the basic values, or unity, and the ratios were determined with reference to these, but it seemed a more accurate method to use the adult percentages for the basic values. These adult percentages were the averages of 52 specimens for each sex and there were only a relatively few cases at the beginning of the fetal period. There was a surprising

TABLE 5

RATIOS OF PERCENTAGE WEIGHTS AT DIFFERENT BODY WEIGHTS. ADULT MALE PERCENTAGES TAKEN AS UNITY. DIGESTIVE TUBE LENGTHS ARE RATIOS TO BODY LENGTH. THESE DATA ARE SHOWN PLOTTED ON BODY WEIGHT IN FIGURES 2 AND 3.

Body Weight	Skeleton	Testes	Dig. tube length	Spleen	Musculature
0.3	1.37	1.62	0.13	0.17	0.42
10	1.05	0.74	0.54	0.42	0.56
20	1.01	0.40	0.63	0.54	0.60
30	0.94	0.31	0.65	0.61	0.61
40	0.93	0.29	0.67	0.64	0.61
50	0.87	0.27	0.69	0.67	0.61
60	0.87	0.21	0.71	0.73	0.64
70	0.87	0.19	0.75	0.80	0.66
80	0.87	0.19	0.80	0.85	0.67
90	0.87	0.19	0.81	0.87	0.66
100	0.87	0.18	0.83	0.85	0.64
110	0.87	0.16	0.84	0.80	0.63
120	0.87	0.16	0.86	0.76	0.62
130	0.87	0.16	0.87	0.72	0.62
140	0.87	0.16	0.89	0.68	0.61
150	0.87	0.16	0.90	0.66	0.61
160	0.87	0.16	0.92	0.63	0.60
170	0.87	0.16	0.93	0.60	0.59
180	0.87	0.16	0.95	0.57	0.59
190	0.87	0.16	0.96	0.53	0.58
200	0.87	0.16	0.97	0.51	0.58
Adult	1.00	1.00	1.00	1.00	1.00

TABLE 6

RATIOS OF PERCENTAGE WEIGHTS AT DIFFERENT BODY WEIGHTS. ADULT MALE PERCENTAGE TAKEN AS UNITY FOR ALL BUT UTERUS. THE RATIOS OF THE THYMUS WERE BASED ON THE INITIAL PERCENTAGE. THESE RATIOS ARE SHOWN PLOTTED ON BODY WEIGHT IN FIGURE 4.

Body Weight	Skin	Kidneys	Uterus	Dig. tube weight	Pancreas	Lungs	Thymus
0.3	0.41	0.16	——	0.15	——	0.99	1.00
10	0.75	0.76	0.51	0.44	0.42	3.25	3.50
20	0.97	0.85	0.52	0.52	0.54	3.40	5.00
30	1.08	0.88	0.52	0.57	0.61	3.26	6.10
40	1.16	0.92	0.53	0.61	0.63	3.14	7.00
50	1.22	0.94	0.58	0.65	0.65	3.00	7.88
60	1.31	0.98	0.62	0.71	0.68	2.88	8.66
70	1.42	1.00	0.67	0.78	0.71	2.76	9.16
80	1.49	1.04	0.73	0.83	0.73	2.65	9.30
90	1.49	1.07	0 80	0.86	0 73	2.52	9.30
100	1.49	1.10	0.88	0.88	0.77	2.47	9.30
110	1.49	1.13	0.97	0.91	0.80	2.47	9.30
120	1.49	1.17	1.11	0.93	0.84	2.47	9.30
130	1.49	1.21	1.25	0.96	0.87	3.65	——
140	1.49	1.24	1.33	0.99	0.91	3.80	——
150	1.49	1.27	1.41	1.01	0.95	3.21	——
160	1.49	1.30	1.47	1.03	0.99	2.99	——
170	1.49	1.34	1.53	1.06	1.02	2.84	——
180	1.49	1.49	1.59	1.09	1.06	2.72	——
190	1.49	1.83	1.66	1.11	1.10	2.60	——
200	1.49	2.17	1.72	1.14	1.13	2.60	——
Adult	1.00	1.00	1.00	1.00	1.00	1.00	——

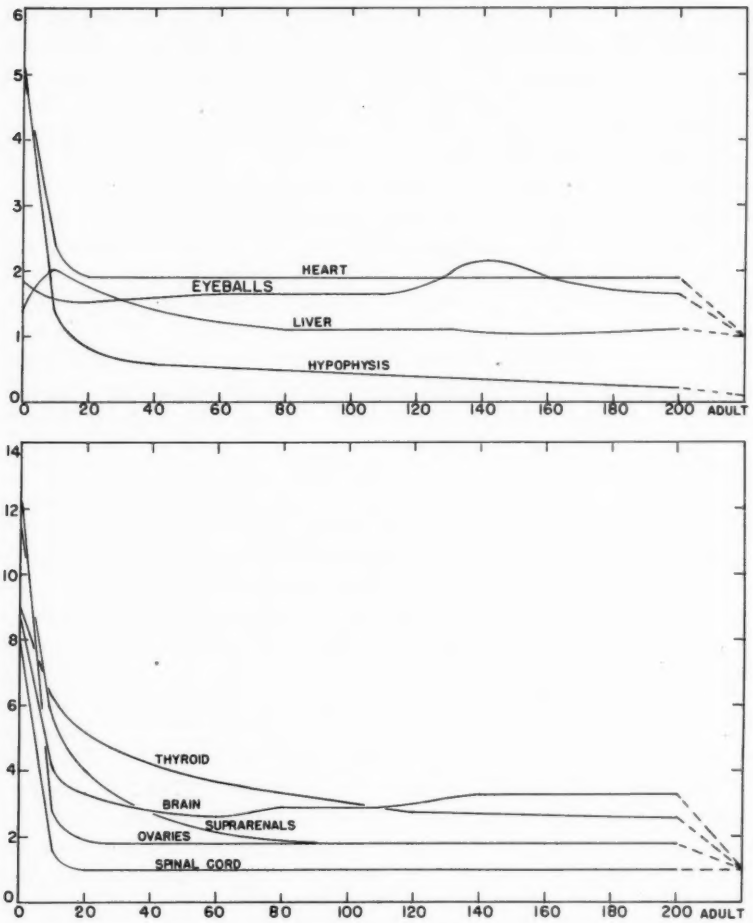
similarity between the two sets of curves. There was a somewhat greater range in the curves using the initial part of the fetal period as unity. The tables and the curves, using the initial fetal percentages as unity, are not shown.

This method has been used by Donaldson in 1923 in the study of the postnatal growth of the various organs in the albino rat, and by Latimer, (1925) for the postnatal growth of the chicken. It seems to serve as well for the fetal growth of the cat and makes the study of the changes in the various organs much easier than a direct comparison of the percentages. The growth of the various organs in absolute weight and in percentage weight have been given in the preceding numbers of this series of the growth of the fetal cat. Each organ does not actually grow independently of the other organs and of the entire body, for each organ is dependent to a greater or less degree upon at least some of the other organs for its normal develop-

ment. The percentage weights give possibly a better picture of the growth of these organs than the absolute weight changes. Naturally the growth of a major part of the body such as the musculature does tend to modify the percentage values of a smaller organ, but these percentage weights give the relative value of each organ or part as far as its weight is concerned.

The nine organs which decrease in relative weight from the beginning of the fetal period to the adult are shown in Table 4, and in the two panels of Figure 1. In all of these, except the ovary, the percentage weight of the organ in the adult male was taken as unity and these ratios were computed for each ten grams increase in body weight. The line connecting the heaviest newborn (200 grams) and the adult, shown at the right, is a dotted line for, so far as is known, there are no data on the changes in this period. Only the weights in the newborn and again in the adult are available. In the lower panel of Figure 1, the ratios given in the left margin show the multiples of the percentage to that in the adult. In the upper panel this is true for all of the organs except the hypophysis. The numbers in the left margin must be multiplied by ten for the correct ratios for the hypophysis.

The maximum percentage for the liver occurs at ten grams of body weight rather than at the beginning of the fetal period. In all other respects the liver follows the general trends of the organs in this group. The curve for the eyeballs is very irregular, as is the percentage curve shown in the earlier paper (Latimer, 1938a), but the total change is not as great as in most of the other organs in this group. It was included here because, with the exception of this postnatal increase, it follows the general plan of this group of organs. The initial size of the eyeballs is probably due to the precocious embryonic development of the eyeballs. The rapid increase at birth may be due to the rapid growth which precedes the beginning of the use of the eyes. The spinal cord changes the least of all of these organs, in the latter part of the fetal period and also in postnatal life. From 20 grams of body weight through the newborn, its percentage weight is approximately the same value as that in the adult. The line for the hypophysis appears to decrease but little, but the ordinates for this organ are ten times as large as for the others and so an apparently small change is really larger. The percentage weight of the hypophysis at 200 grams of body weight is 2.06 times the percentage in the adult.



DESCRIPTION OF FIGURES

FIG. 1. Curves showing the percentage changes as multiples of the percentage in the adult male (female for the ovaries), taken as unity. The abscissae represent the body weight in grams and at the right, the adult male body weight. The ordinates show the number of times the percentage is greater than the percentage in the adult male. The numbers in the left margin show exact numbers for all organs but the hypophysis. For the hypophysis the numbers represent multiples of ten.

The greatest change in these ratios for any of the organs is shown in Table 4, for the hypophysis. Table 1 shows that the hypophysis increases but 6.4 times in the postnatal period in the male while the body weight is increasing 23.5 times. The ratios for the suprarenals and the ovaries are identical from 90 grams of body weight through the newborn and also in the adult. This does not mean that they form the same percentage of the body, but merely that they have the same ratio to the percentage in the adult male. In fact the percentage weight of the suprarenals is greater than that of the ovaries. The values for the suprarenals are 0.025 per cent in the newborn and 0.014 per cent, in the adult male. The similar percentages for the ovaries are respectively, 0.016 and 0.009 per cent.

The ratios of the percentage weights to the percentages in the early fetal period were determined and plotted as in Figure 1, and all of the curves thus obtained were very like those shown in Figure 1 and so they are not given here. The greatest difference was in the liver. The rise to the maximum at ten grams of body weight was much more pronounced but the general trend of the curve in all other respects was similar to the one shown in Figure 1.

All of these organs, except the spinal cord, are found in the lower divisions of Tables 1 and 2, or with a total increase less than that for the body weight. The spinal cord has a slightly greater increase in weight in the postnatal period than the total body weight. There is a slight increase in the ratios of the weight of the brain in the latter part of the fetal period and in the newborn. This increase is not large and the general trend of the growth seemed to warrant the listing of the brain in this group, for in general its changes in percentage weight follow the plan found in the other organs in this group.

A comparison of the ratios for the postnatal growth of the rat and the chicken shows that in general the dotted lines in Figure 1, connecting the newborn and the adult, give about the correct nature of the postnatal change. Donaldson finds that the ovary has the same relative weight in the newborn and again at 50 and at 400 grams of body weight with a maximum at 100 grams of body weight. The liver has a postnatal maximum soon after birth in both the rat and chicken. In the chicken it decreases to its minimum in the adult, but in the rat it has the same relative weight in the adult and in the newborn. The heart, brain, spinal cord and suprarenals decrease from birth to ma-

turity in the chicken but in the rat they have a slight rise immediately after birth, and then decrease to their minimum. In the chicken the heart increases slightly in the older chickens but it still remains below the percentage of the newborn. In the rat it has its minimum in the oldest rats.

Eight of the nine organs shown in Figure 1, or the hypophysis, brain, cord, eyeballs, heart, suprarenals, thyroid and ovaries decrease from a maximum percentage of the body weight at the beginning of the fetal period to their minimum in the adult. The maximum for the liver occurs at 10 grams of body weight and then it too decreases to its minimum in the adult. In other words, all of these, except the eyeballs, develop early and then grow more slowly in the late fetal period and also in the postnatal period. The eyeballs have their maximum in the newborn but their total growth, with this one exception, is like the other organs in this group. The weight of the spinal cord tends to follow most closely the body weight in the latter part of the fetal and also in the postnatal growth.

There are but two parts in the second group, or the skeleton and the testes. The ratios for these are shown in the first two columns of Table 5, and the curves in Figure 2. These, like the organs shown in Figure 1, decrease in the fetal period but they increase in the postnatal period and in this respect they are different from the nine organs shown in Figure 1. Their maximum percentage, like those in the preceding group, is found at the beginning of the fetal period. The skeleton

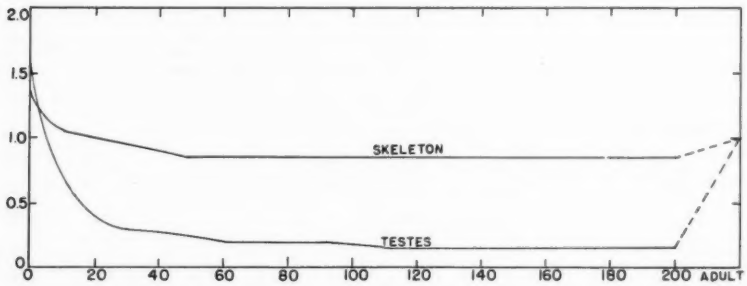


FIG. 2. Curves showing relative percentage changes as multiples of the percentage weight in the adult male. The abscissae represent body weight in grams, with the average body weight of the adult males shown at the extreme right. The ordinates give multiples of the adult percentage weight.

follows the growth of the entire body more closely than do the testes. Both have an increase in absolute weight less than that of the total body weight in the prenatal period and more than the total body weight in the postnatal period (Table 1). Their total increase in absolute weight, as shown in Table 2, is slightly less than the total body weight increase. The postnatal growth of the chicken skeleton has its maximum in early postnatal life and then decreases to its minimum in the adult. Jackson and Lowrey (1912) show the skeleton of the albino rat increasing to its maximum at one week of postnatal life and then decreasing steadily to maturity. The rat testes increase to their maximum at 100 grams of body weight (Donaldson, 1923) and then decrease to the percentages found in the adult. Their percentage weight in the adult albino rat is about 9.5 times the percentage at birth.

In general the skeleton and the testes of the cat are relatively heaviest in the early fetus, decrease to their minimum in the newborn and then rise slightly to the percentage found in the adult but still less than the initial percentage.

The third group is composed of the weights of the musculature and the spleen and the length of the digestive tube. These, as shown in the last three columns of Table 5, and in Figure 3, have their minimum percentages at the beginning of the fetal period and their maxima in the adult. The prenatal course of development is different from that shown in the preceding two figures, for these increase during the prenatal period. One of them, the length of the digestive tube increases throughout the entire prenatal and also the postnatal period without any decrease. The other two, or the musculature and the spleen have a secondary maximum shortly before birth and then at birth their percentages drop slightly, with a marked increase in the postnatal period to the maximum in the adult.

The spleen and the musculature have a greater increase in the prenatal and in the postnatal periods (Table 1) than the total increase in the body weight, and also in the total increase, shown in Table 2. The length of the digestive tube is close to the bottom of the total increases shown in Tables 1 and 2. It is the only linear dimension in this study, and linear dimensions, although maintaining their relative positions with ponderal measurements, have smaller increases. Hence, it is rather unfair to compare this one linear dimension with the other ponderal dimensions.

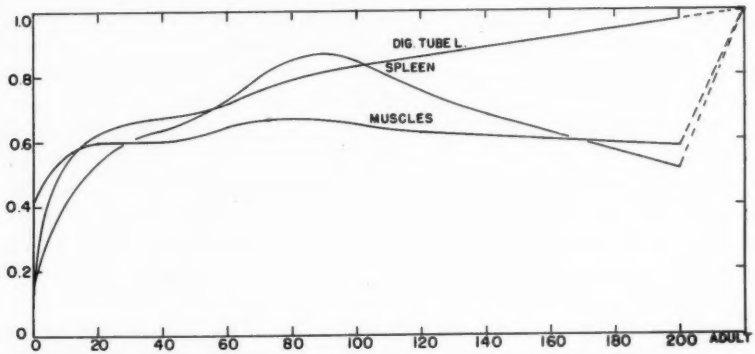


FIG. 3. Curves showing the percentage changes as multiples of the percentage in the adult male. The abscissae represent the body weight in grams and at the right, the adult male body weight. The ordinates show the ratios to the adult male percentage weight or length taken as unity.

The relative postnatal growth of the muscles of the chicken and of the rat (Jackson and Lowrey, 1912) increase to their maximum in the adult. In both the rat and the chicken the relative weight of the spleen is greater in the adult than in the newborn but in both of these the maximum percentage comes shortly after birth and is followed by a decrease, but not as far as the percentage level of the newborn. Briefly, the musculature and the spleen of the cat rise from their minimum at the beginning of the fetal period to a secondary maximum in the late fetal period, drop slightly at time of birth and then attain their maximum in the adult, and the length of the digestive tube increases continuously from the beginning of the fetal period to the adult.

The relative growth of the fourth and the last group of these organs is shown in Table 6 and in the two panels of Figure 4. The ordinates in the left margin in both panels give the actual ratios for all of the parts except for the thymus and the lungs. The ratios for these two organs should be ten times the ratios shown in the figure. In all but two of these, the percentage weight in the adult male was taken as unity for the computations of these ratios. For the thymus the initial percentage at the beginning of the fetal period, was used as unity and for the uterus the percentage in the adult female was used. All but two of these organs have their minima at the beginning of the fetal

period, rise to their maxima at birth and then decrease somewhat to the percentage found in the adult. The two exceptions are the lungs which have the same relative weight in the early fetal and in the adult stage, and the thymus which is shown only in the fetal period. It was so small in most of the adult cats that it was not located nor weighed.

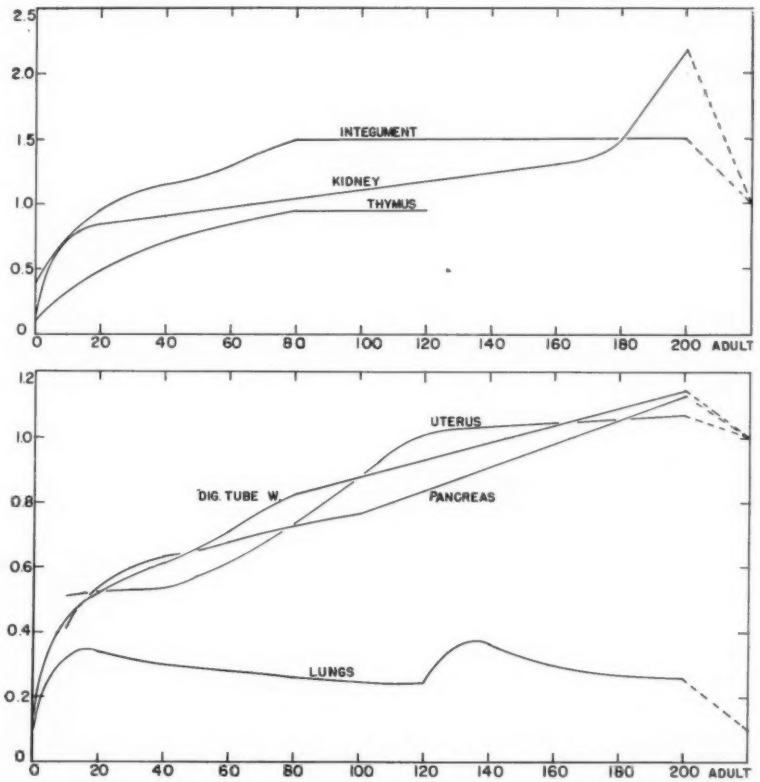


FIG. 4. Curves showing the relative percentage changes as multiples of the percentage weight in the adult male taken as unity, except female for uterus. The abscissae represent the body weight in grams and at the right the weight of the adult male. With two exceptions the ordinates in left margin show the ratios of the percentage in the adult. The percentage weight of the thymus in early fetal life is taken as unity, and the ordinates for the thymus and the lungs are ten times the units shown in the left margin.

The percentage weight of the thymus at the beginning of the fetal period, obviously, was taken as unity for the computation of the ratios shown in the upper panel of Figure 1 and Table 6. It, like the others in this group, increases to its maximum at birth, or very likely at some time following birth. It was put in this group for its growth, so far as it was available for these cats, followed the plan of this group. It is shown with the greatest increase in absolute weight of any of the organs in the fetal period (Table 1). The lungs have an irregular course, rising to a secondary maximum shortly after the onset of the fetal period, then slowing in their growth rate somewhat in the latter part of the fetal period, then rising rapidly to their maximum percentage weight shortly after birth, and then again decreasing to unity or the same relative weight in the adult that they had at the start of the fetal period. Very likely a separate group should have been made for the lungs, but they were put in this group for they seemed to fit in best with these organs.

The total increase in the weights of the integument, kidneys and the weight of the entire digestive tube is greater than the total body weight increase, while the total increase of the uterus, pancreas, and the lungs is less than the total body weight increase, as shown in Table 2. If the position of these organs is studied in Table 1, it is seen that the integument, kidneys, lungs, digestive tube weight and the thymus are in the upper group, or those organs which increase more than the total body weight increase in the fetal period, and all but the thymus, which is not shown in the postnatal growth, and the weight of the digestive tube, grow less than the total body weight in the postnatal period. The postnatal increase in the weight of the digestive tube is but slightly greater than the increase in the entire body weight. The uterus increases less than the total body weight in both periods. The pancreas and the uterus start their development later in the fetal period than the other organs and because of their small size both of these were not weighed until ten grams of body weight. The pancreas increases less than the total body weight in the fetal period but more than the body weight in the postnatal period. Table 1 shows the total increase for each organ, while Figure 4 shows the position of each organ relative to its adult percentage of the total body weight.

The postnatal growth of some of these organs is not shown for the rat and chicken but all of those shown, namely . . . kidneys, thymus,

weight of the digestive tube, pancreas and the skin in the rat (Jackson and Lowrey, 1912), have maxima at varying times following birth and all but the weight of the digestive tube of the rat ultimately decrease to a point less than the percentage at birth. In the rat the intestinal tract rises and then decreases but remains at a higher percentage than at time of birth. The postnatal growth of the lungs in the rat decreases from its maximum at time of birth. Hall and MacGregor (1937) find that the kidneys in the cat decrease from their maximum at time of birth to the early adult and then increase slightly in the adults of greater body weight. The kidneys are the only organs of the cat for which we have complete data from early fetal life through to the adult stage.

The curves showing the ratios based on the initial fetal weights taken as unity are all very similar to the curves shown in Figures 2, 3 and 4. There is a greater difference for some of the organs shown in Figure 4. The general trends of the curves are the same, but with the fetal weight taken as unity the differences in the percentages of the adult parts are shown better, for example, the ratios for the weight of the digestive tube show that it forms a much larger percentage of the body weight than the pancreas and the uterus. The general shape of the curve is the same, but when the fetal weights are used as unity the curves of the larger parts of the body end at a higher level than the organs which form but small percentages of the adult weight.

In general, the changes in the percentages of the integument, kidneys, uterus, digestive tube weight, and the pancreas are much alike. They all increase in relative weight throughout the fetal period and after attaining a maximum percentage in the newborn, they form smaller percentages in the adult. The thymus is included although its weight in the adult was not determined. The lungs are irregular but they fit best in this group. They have a secondary rise early in fetal life, the maximum at birth and then form the same percentage of the body weight in the adult as they do at the beginning of the fetal period.

Summary

Seven organs and systems increase more than the total body weight in both the prenatal and also in the postnatal periods. There are three systems and organs found in this group in both periods: weight of the digestive tube, muscles and spleen. The four other organs in the prenatal period are: thymus, kidneys, skin, and the lungs. The four additional organs in the postnatal period are: testes, pancreas, skeleton and spinal cord. All of the other organs increase less than the total body weight.

Nine organs have their maximum percentage weights at the beginning of the fetal period. They are, brain, spinal cord, hypophysis, heart, ovaries, thyroid, suprarenals, testes, and skeleton. The maximum percentage of the liver is found shortly after the beginning of the fetal period or at a body weight of ten grams. The lungs, thymus (as far as the available data show), kidneys, integument, uterus, pancreas, eyeballs and the weight of the digestive tube all have their maxima in the newborn. Only three parts have their maxima in the adult, or the spleen, the muscles and the length of the digestive tube.

The organs may be placed in four groups based on their changes relative to their adult percentage weight.

Group I, contains those organs which decrease in relative weight from the beginning of the fetal period to the adult. There are nine organs in this group: heart, liver, hypophysis, thyroid, brain, spinal cord, eyeballs, suprarenals and ovaries.

Group II, decrease from their maximum percentages at the beginning of the fetal period, but these have a slightly higher percentage in the adult than in the newborn. This group consists of the skeleton and the testes.

Group III, contains three systems or organs, the musculature, the spleen and the length of the digestive tube. The first two of these have a secondary maximum in the late fetal period and after birth rise to their maxima in the adult. The length of the digestive tube, the only linear measurement, rises throughout both prenatal and postnatal periods, to its adult maximum.

Group IV contains seven organs and systems. They increase to their maxima in the newborn and then decrease slightly to the per-

centages found in the adult. This group includes, skin, kidneys, weight of the digestive tube, uterus, and the pancreas. Two organs, the thymus and the lungs are included although they are somewhat different from the others.

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GROWTH OF THE LIVER IN FETAL RATS*

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During the course of an investigation of the movement of vitamin A across the placentae of pregnant rats, it became necessary to estimate the fetal liver weight A search of the literature revealed no data on the relationship between liver weight and total body weight in fetal rats, although this relationship has been established for human fetuses (Jackson, 1909), unhatched birds (Kaufman, 1930) and many adult mammals (Brody, 1945). Therefore, it was undertaken to determine the body weight and corresponding liver weight in fetal rats.

Data were obtained from 70 fetuses representing 12 litters (Table I). The maternal and paternal animals were albino rats from the colony of the Harvard Biological Laboratory.

Two males were kept with four females for about one week. Thereafter, the females were kept in separate cages. When the gestation had progressed sufficiently, the pregnant animals were anesthetized with ether, the fetuses removed from the uterus and the fetal livers weighed within a period of one hour. Those fetuses which showed no movements on being taken from the uterus were discarded so that only live fetuses were included. All livers were weighed immediately upon excision from the fetuses in order to keep the loss of weight by evaporation to a minimum.

The young of litters 34 and 91 were born alive. The livers were removed from these young rats within two hours after birth. In no instance was milk found in the stomach.

The numerical data are tabulated in Table I and graphed on a log-log grid in Fig. 1. Since a very large and rapid accretion of weight occurs during the last few days of parturition (Hamilton and Dewar,

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TABLE I

RELATION OF TOTAL WEIGHT TO LIVER WEIGHT IN FETAL ALBINO RATS

Litter#	*Days of Gestation	Fetal Weight	Fetal Liver Weight	Litter#	*Days of Gestation	Fetal Weight	Fetal Liver Weight	Litter#	*Days of Gestation	Fetal Weight	Fetal Liver Weight	Litter#	*Days of Gestation	Fetal Weight	Fetal Liver Weight
		Gms.	mgs.			Gms.	mgs.			Gms.	mgs.			Gms.	mgs.
35	16	1.4	94	40	18	3.1	223	85	20	3.4	239	41	21	4.9	299
		1.3	91			3.0	203			3.5	238			4.7	315
		1.5	112			3.0	185			3.4	222			4.4	309
		1.5	110			3.2	216			4.1	260			5.1	292
		1.5	101			3.3	237			3.6	243				
		1.6	119			3.2	215			3.7	286	91	22	5.5	289
		1.5	124			3.1	209			3.5	232			5.3	325
						3.1	210							5.2	313
32	17	1.8	143					33	20	3.2	189			5.2	275
		1.8	133	86	19	3.2	226			3.8	252			5.6	308
		1.9	161			3.4	228			3.2	233				
		2.1	152			3.6	221			3.2	228	34	24	6.1	307
		2.1	165			3.6	197			3.4	219			5.9	295
		2.1	157			3.6	241			3.5	212			5.4	308
										3.7	255			5.6	290
90	18	2.0	157	84	19	3.2	216							6.0	306
		2.1	155			3.1	230	31	21	4.5	282			5.8	291
		2.1	176			3.6	253			5.3	305			5.5	296
		2.2	174			3.2	227			5.2	314			5.2	309
		2.2	155												
		2.3	181												

*Estimated.

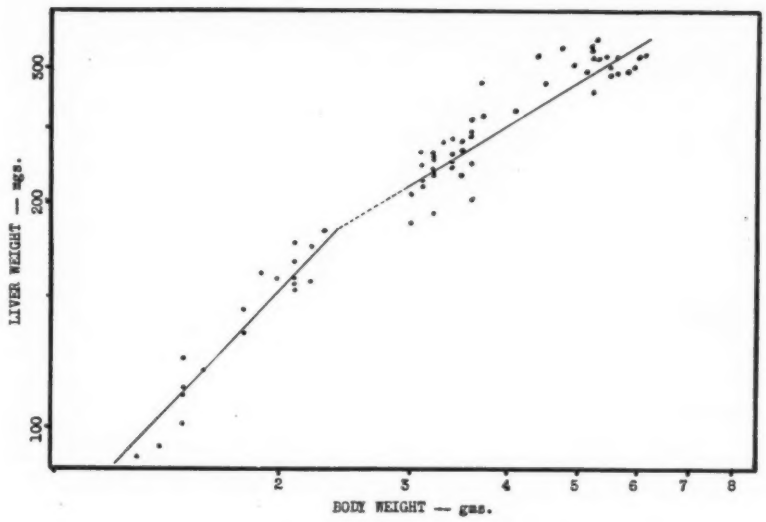

FIG. 1
THE RELATIONSHIP BETWEEN TOTAL BODY WEIGHT AND LIVER WEIGHT IN FETAL RATS.

1938), it does not seem unreasonable to expect to find a point of flexion on the graph. The equation for the first part of the line (representing the fetuses weighing from 1.3 to 2.3 gms) is:—

$$Y = 72\,X^{1.07}$$

where X is the body weight in grams, and Y, the liver weight in milligrams.

The following equation represents the line for fetuses of 3 gms. and over:—

$$Y = 106\,X^{0.61}$$

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INCREASE IN WEIGHT AND GROWTH OF CHILDREN IN THE FIRST YEAR OF LIFE*

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In this paper an attempt has been made to derive on the basis of physical and physiological conceptions the relationship of weight and growth of children in early life, i.e., approximately until the first attempts are made at walking.

Besides the energy balance which governs the course of weight and growth from a physical standpoint, in order to solve our problem it is still necessary to make some assumptions which can be justified from conceptions and also from observations.

These assumptions are:

I. On the average during the growth of the child, stature and organs are geometrically similar. This statement is of course valid only as an approximation for the relatively short time of the first year of life.

II. Food which the healthy child eats and digests is always proportional to the surface area of the bowel.

III. The amount of heat transmitted from the child to the surrounding is proportional to the surface area of the body and the lungs.

IV. At every stage of growth the stress of muscles in controlling the various internal organs is the same or the work of muscles in volume units is invariable. This assumption follows from the conception that nature, like an architect, always uses muscle-material in such a way that there is neither an over—nor an under—stress of the muscles. That the stress of the muscles is invariable is also valid as an approximation only for the relatively short time of the first year of life.

*This research arose towards the end of 1944 in the "Krankenbau" of the Birkenau-Auschwitz concentration camp, and was inspired by Prof. B. Epstein of Prague, who, a prisoner too, was in charge of the "Krankenbau." Prof. Epstein had already discovered before the war, on the basis of statistical material, that the weight of a child in its first year of life depends on its initial weight and its initial rate of increase of weight.

V. The working rhythm of the various organs of the body in the first year of life is essentially the same.

VI. The increase in weight of the child is the greatest immediately after its birth, or more precisely, we assume that time is estimated from the moment when increase is maximum.

The agreement between the theoretically obtained results and the statistically calculated mean values of weight proves of course only over the whole range, the validity of the above enumerated assumptions.

If l, f, v, represent a certain length, surface area and volume of the child's body at time t, and l_0, f_0, v_0 the corresponding measurement at the beginning of growth at the time $t = O$, then if λ is an average linear coefficient of growth at the time t, we know on the basis of assumption I, that

$$l = \lambda.l_0\,, \quad f = \lambda^2.f_0, \quad v = \lambda^3.v_0 \qquad 1/\text{a, b, c.}$$

If V_0 is the total volume of the child's body at time $t = O$ and V its volume at time t, then from 1/c also

$$V = \lambda^3.V_0\,. \qquad 2/$$

Multiplying 2/ by the average specific weight of the body, then

$$G = \lambda^3.G_0\,, \qquad 3/$$

where G is the child's weight at time t and G_0 at time $t = O$. Eliminating λ from equations 1/ by help of 3/ we have

$$l = l_0\left(\frac{G}{G_0}\right)^{1/3}, \quad f = f_0\left(\frac{G}{G_0}\right)^{2/3}. \quad v = v_0\left(\frac{G}{G_0}\right). \quad 4/\text{a, b, c.}$$

On the basis of assumption II in its relation to equation 4/b the amount of food N in calories digested by the child is proportional to $G^{2/3}$, hence

$$N = n.G^{2/3}. \qquad 5/$$

From assumption III and equation 4/b the amount of heat W in calories transmitted from the child to the surroundings is also proportional to $G^{2/3}$, hence

$$W = w.G^{2/3}. \qquad 6/$$

By the help of condition IV and equation 4/c we have finally the work of muscles M in calories necessary to control the body organs proportional to G, hence

$$M = m.G. \qquad 7/$$

We may suppose that not only the intake of food but also the work of the muscles of the body organs is continuous, neglecting time oscillations. Then using assumption V, we obtain the energy balance, where dG is the increase in the child's weight in the time dt and $c.dG$ is the number of calories corresponding to the increase in weight:

$N.dt =$		$W.dt$	$+$	$M.dt$	$+$	$c.dG$	8/
Energy absorbed		Energy transmitted to the surroundings		Energy used for the work of the muscles		Energy stored by increase of weight	

and together with equations 5/, 6/, 7/ gives

$$\frac{dG}{dt} = \dot{G} = \frac{1}{c}(N - W - M) = \frac{n - w}{c} G^{2/3} \frac{m}{c} G$$

Introducing new constants $a = \frac{n - w}{c}$., $b = \frac{m}{c}$

$$\dot{G} = a.G^{2/3} - b.G\,, \qquad 9/$$

$$\ddot{G} = \frac{2a}{3} G^{-1/3} - b \;.\dot{G}\,. \qquad 10/$$

From 9/ we get

$$dt = \frac{dG}{a.G^{2/3} - b.G}$$

and by integration

$$t = -\frac{3}{b} ln(a - b.G^{1/3}) + K . \qquad 11/$$

Constants a, b and integration constant K are determined by the initial conditions. For $t = 0$, *1*: $G = G_0$ and *2*: $G = \dot{G}_0$. According to assumption VI, $\dot{G}$ is maximum for $t = 0$, then *3*: $\ddot{G}_0 = 0$. From equation 10/ and relationship *1* and *3* above

$$a = \frac{3}{2} b.G_0^{1/3}.$$

Using this relationship with conditions *1* and *2* we have from equation 9/

$$b = 2\frac{\dot{G}_0}{G_0}.$$

Thus

$$a = 3\frac{\dot{G}_0}{G_0^{2/3}}$$

Equation 11/ with condition *1* gives

$$K = \frac{3}{b} ln\,(a - b.G_0^{1/3})$$

Inserting values for constants a, b, K in equation 11/

$$t = -\frac{3G_0}{2\dot{G}_0} ln\left[3 - 2\left(\frac{G}{G_0}\right)^{1/3}\right] \qquad 12/$$

Solving equation 12/ for G we have

$$G = \frac{G_0}{8}\left[3 - \epsilon^{-\frac{2\dot{G}_0}{3G_0}t}\right]^3 \qquad 13/$$

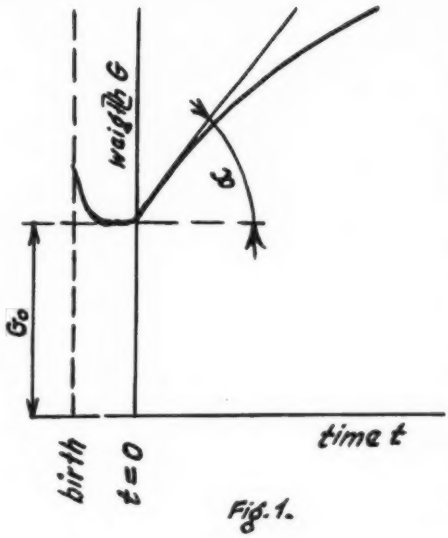

Fig. 1.

This is the formula sought for, giving the relationship between weight and time where G_0 is the initial weight and $\dot{G}_0$ the initial increase of weight.

To show quite clearly from which moment we begin to count the time and what G_0 and $\dot{G}_0$ mean, we show schematically in figure 1 the change of weight immediately after birth. We begin to count the time /t = 0/ after posturale decrease of weight and G_0 is the weight of the child and $\dot{G}_0 = \text{tg}\alpha$ the gradient of the tangent to the change of weight in this moment.

From 1/a and $4/a\lambda = \left(\frac{G}{G_0}\right)^{1/3}$ and using equation 13/ we get the average linear coefficient of growth

$$\lambda = \frac{1}{2}\left[3 - e^{-\frac{2\dot{G}_0}{3G_0}t}\right] \qquad 14/$$

As in reality the linear coefficient of growth is not the same in all directions, the average linear coefficient of growth is only a fiction and cannot be controlled by measurement as it is possible for the weight.

In table 1, the values of weights according to equation 13/ for the initial values $G_0 = 3200\ g$ and $\dot{G}_0 = 100, 150, 200, 250, 300 g$/weekly are calculated. Diagram I shows graphically the change in weight according to table 1. Table 2 gives the average linear coefficient of growth according to equation 14/ for the same initial values. In

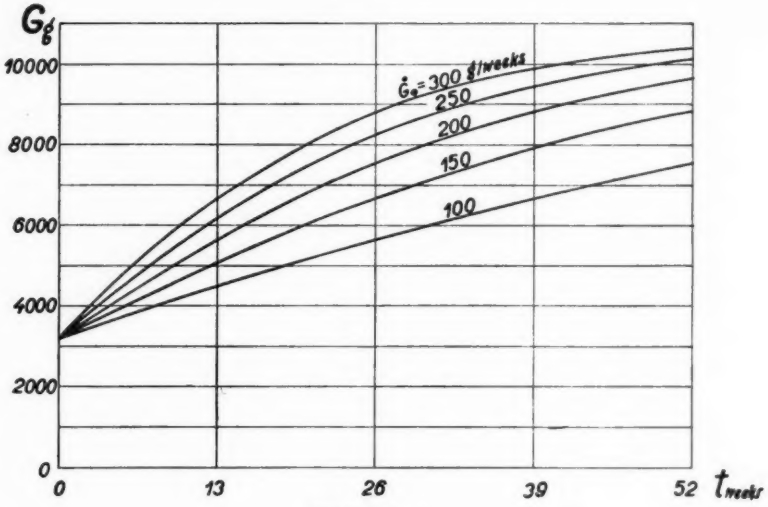

DIAGRAM I.

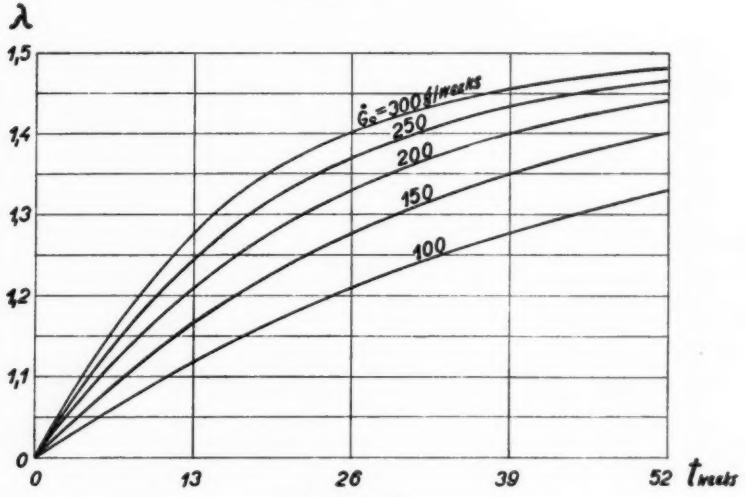

DIAGRAM II.

TABLE 1. (G).

$\dot{G}_o \backslash t$	0 weeks	13 weeks	26 weeks	39 weeks	52 weeks
100 g/weekly	3200 g	4479 g	5656 g	6681 g	7541 g
150 "	3200 "	5085 "	6681 "	7912 "	8810 "
200 "	3200 "	5656 "	7541 "	8810 "	9609 "
250 "	3200 "	6190 "	8245 "	9444 "	10096 "
300 "	3200 "	6681 "	8810 "	9884 "	10387 "

TABLE 2. (λ).

$\dot{G}_o \backslash t$	0 weeks	13 weeks	26 weeks	39 weeks	52 weeks
100 g/weekly	1	1,119	1,209	1,278	1,331
150 "	1	1,167	1,278	1,352	1,402
200 "	1	1,209	1,331	1,402	1,443
250 "	1	1,246	1,371	1,434	1,467
300 "	1	1,278	1,402	1,456	1,481

Diagram II is shown the variation in the linear average coefficient of growth according to table 2.

The average statistical values of increase in weight of children in the first year of life, communicated to the author by Prof. B. Epstein, agree well with the theoretically calculated values. This agreement encouraged the author, who is a layman in this field, to place his deductions before the experts.

Summary

The law of energy in conjunction with some ingenious assumptions on the growth procedure of the child in the first period of life leads to a mathematical formula for the increase of weight of the child during its first year. This formula comprises no empirical constants. Therefore, its validity is not restricted to the growth of children. It might be of practical value for the pediatrician and of scientific interest for the biologist.

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Growth, 1948, **12**, 157-163.

GROWTH REGULATING ACTION OF SALICYLACETONE[1]

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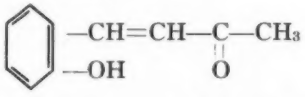
Growth regulating action of diverse compounds has been correlated recently with the presence in the molecule of a substituted vinyl ketone group, $-\overset{3}{\underline{CH}}=\overset{2}{\underline{CH}}-\overset{1}{\underset{\underset{O}{\|}}{\underline{C}}}-$, such as occurs in mesityl oxide, penicillic acid, and clavacin (Geiger and Conn, '45). The physiological activity of these compounds may be correlated with the possibility of addition of R—SH on the double bond. We have found a compound of this type with an *o*-hydroxyphenyl substitution on position 3, namely salicylacetone, to possess marked ability to influence growth.

Salicylacetone, 4(*o*-hydroxyphenyl)-3-butenone-2, was prepared by one of us according to the method described by Harries ('91). Several structural formulae have been assigned to this compound due to the assumed possibility of ring formation; on the basis of published spectrographic analysis of related compounds (Bodforss, '38; Wilds, *et al.*, '47) we favor the suggestion that the following structure exists in neutral and weakly acid media:

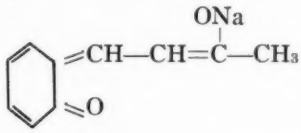


(1)

Depending upon the intended use, in the experiments described below, stock solutions of the active compound were prepared either in a 70/30 mixture of 95 per cent ethyl alcohol and acetone or in aqueous

[1]This work was supported in part by a generous research grant from the Cutter Laboratories, Berkeley, California.

1N NaOH. The fact that the absorption spectrum of the sodium salt is shifted into the visible region suggests the orthoquinoid structure for this salt as follows:



(2)

Experiments and Results

1. *Action on fungi.*—Fungistatic assays were made by the agar cup plate technique on Sabouraud's medium seeded with *Trichophyton mentagrophytes* 162-1501[2], and incubated at 26°C. and approximately 65 per cent relative humidity. The central wells into which solutions of salicylacetone in the 70/30 alcohol-acetone mixture were placed were 1 cm. in diameter. The solvent exerted no inhibitory action. Averaged data plotted in figure 1 show that inhibition of growth was approximately a logarithmic function of concentration over the range from 25 to 5,000 mg./L. The upper and lower curves represent average diameters of zones of inhibition measured after five days and seven days, respectively. From the fifth to the seventh day the curve is shifted downwards due to infringement on the zone by aerial hyphae from uninhibited peripheral regions. No further decrease in zone diameter occurred after the seventh day.

Incorporation of salicylacetone in Sabouraud's medium completely inhibited growth of *T. mentagrophytes* at a concentration of 100 mg./L. and very markedly reduced growth at a concentration of 10 or 1 mg./L. Similarly, complete inhibition occurred when small amounts of the crystals were placed at the center of the plates following a modified technique based on the procedure described by Brewer ('39). This technique applied to plates seeded with *Botrytis allii*[3] produced zones 7.0 cm. in diameter in three days. There was no decrease in diameter after this time. When tested by the same technique. Desenex, a preparation of zinc undecylenate and undecylenic acid, produced zones 4.1 cm. in diameter on plates seeded with *T. menta-*

[2]Number assigned to this strain in the collection of the Tropical Diseases Laboratory of the University of California Medical Center.

[3]This plant pathogen has been introduced as a test organism in fungicidal studies by Dr. P. W. Brian, Imperial Chemical Laboratories, Ltd., through whose courtesy it was made available to us.

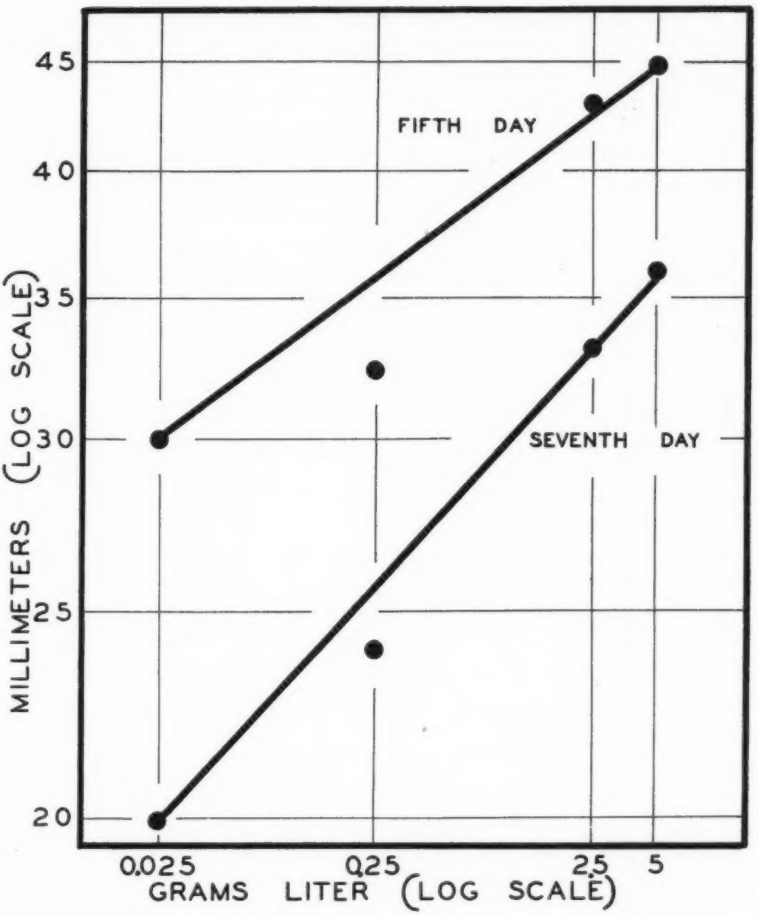


FIG. 1.

grophytes and no inhibition against *B. allii*; the latter organism grew up to and over the compound.

2. *Action on root formation.*—Although salicylacetone in the concentrations that were employed inhibits the growth of *T. menta-*

grophytes and of *B. allii,* it stimulates formation and early growth of roots in appropriate varieties of potato (*Solanum tuberosum*)[4], of *Ficus gnaptholocarpa*[5], and probably of other plants.

Potato tubers were cut into sections each of which contained a single "eye". These were placed in aqueous solutions of salicylacetone ranging from 30 to 200 mg./L. in 0.02 M. phosphate buffer at pH 6.9. The control for each solution consisted of a similar section cut from the same region of the tuber and placed in buffer solution at the same pH. The varieties De Soto and Yampa were the most useful of the several that were used. The results shown in table 1 are from a typical experiment and illustrate the stimulation in rooting induced by two concentrations of salicylacetone.

TABLE 1

FREQUENCY OF OCCURRENCE OF ROOTS OF DIFFERENT LENGTHS PER BUD OF *Solanum tuberosum* (VAR. DE SOTO) IN 0.02 M. PHOSPHATE BUFFER AT PH 6.9 AND IN BUFFER PLUS SALICYLACETONE

Figures are for observations made after four days at 26°C.

Length of roots, cm.	Average[a] number of roots in Salicylacetone		
	Buffer	100 mg./L.	200 mg./L.
<0.5	12	16	14
0.5 to 0.9	1.3	7	8
1.0 to 1.4	0.3	5	5
1.5 to 1.9	none	3	2
2.0 to 2.4	none	1	0
2.5 to 2.9	none	0.3	1.5
3.0 to 3.4	none	0.3	0.3
3.5 to 3.9	none	0.6	0.6
4.0 to 4.4	none	0.3	0.3

[a]Based on observation of six sections.

Three cuttings of *Fiscus gnaptholocarpa*, each embodying 6 nodes, were placed in each of a number of 1-liter Erlenmyer flasks containing 150 ml. of a solution of salicylacetone (4 to 150 mg./L.) in distilled water or in 0.02 M phosphate buffer. Thus only the lower extremity of each cutting was immersed. Controls consisted of similar cuttings in distilled water or in phosphate buffer. The cuttings each possessed several buds but no leaves or roots. Experiments were performed at pH 5.5 and at pH 7.0.

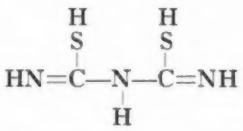
[4]We wish to acknowledge the courtesy of Dr. T. P. Dykstra, United States Department of Agriculture, Baton Rouge, Louisiana, and of Dr. W. E. Pike, Colorado A. and M. College, Fort Collins, Colorado, in providing us with tubers of several different varieties of potato.

[5]Kindly provided by Dr. I. J. Condit, University of California Citrus Experiment Station, Riverside, Californa.

Control cuttings with the bases immersed in distilled water or in plain phosphate buffer at pH 5.5 or 7.0, and those in salicylacetone at 4 mg/L. at pH 7.0, developed root initials, and the buds swelled but failed to develop leaves: the cuttings died within 3 weeks. The most evident growth activating effect was obtained with salicylacetone in concentrations of 10 to 50 mg./L. either at pH 5.5 or pH 7.0. Root initials formed within a few days, then the leaf buds expanded and the leaves which developed apparently synthesized materials that promoted growth of roots from the induced root initials: higher concentrations up to 100 mg./L. were well tolerated. As the concentration was increased, the polarity gradient of the cuttings lessened. This was evidenced by development of root initials further from the basal end. Conversely, buds grew from nodes further removed from the apical end. In the controls containing only distilled water or phosphate buffer, the buds began to swell but later wilted and the cuttings died without any root initial having elongated.

It is worthy of comment in passing that salicylacetone promoted formation of chlorophyll, and, consequently, survival of excised tissues and twigs. This was observed in sections of potato tubers and of fig twigs. Growth of shoots was noticeably increased in the potato experiments, while chlorophyll developed markedly in the thin bark in the basal portions of fig cuttings immersed in salicylacetone, but failed to develop in the controls.

The fact that the activity of such compounds as salicylacetone may be correlated with possible addition of R-SH on the double bond, suggested studying the interaction with salicylacetone of a compound containing 2 -SH groups such as dithiobiuret[6], to which Preisler and Bateman, ('47) have assigned the formula



(3)

This compound which appears to possess growth-stimulating properties for shoots (see table 2) was found to act synergistically with salicylacetone in promoting development of roots. A concentration of

[6]Research sample kindly provided by the American Cyanamid Company, New York, New York.

TABLE 2

DEVELOPMENT OF LEAFY SHOOTS FROM BUDS AT DIFFERENT NODES ON CUTTINGS OF *Ficus gnaptholocarpa* IN SOLUTIONS OF SALICYLACETONE, DITHIOBIURET, DISTILLED WATER, OR PHOSPHATE BUFFER, AND IN COMBINATIONS OF THESE

Salicylacetone, mg./L.	Medium	Average number of shoots developed from node[a] 1	2	3	4	5	6
150	Dithiobiuret, 20 mg./L. in distilled H_2O	3	3	3	3	—	—
100	Distilled H_2O	3	—	—	—	—	—
33	Dithiobiuret, 20 mg./L. in distilled H_2O	3	3	3	—	—	—
	Distilled H_2O	1	—	—	—	—	—
11	Dithiobiuret, 20 mg./L. in distilled H_2O	2	2	2	1	—	—
	Distilled H_2O	3	3	3	1	—	—
	Phosphate buffer, pH 5.5	3	3	1	—	—	—
	Phosphate buffer, pH 7.0	—	—	1	2	—	—
4	Distilled H_2O	3	—	—	—	—	—
	Phosphate buffer, pH 7.0	3	—	—	—	—	—
0	Dithiobiuret 20 mg./L. in distilled H_2O	3	2	3	2	—	—
	Dithiobiuret, 75 mg./L. in distilled H_2O	3	2	3	2	—	—
	Phosphate buffer, pH 5.5	1	—	1	—	—	—
	Phosphate buffer, pH 7.0	1	1	2	—	—	—

[a]Nodes are numbered from apex to base

100 to 150 mg. of salicylacetone and 20 mg. of dithiobiuret per liter effectively stimulated development of buds into leafy shoots which apparently synthesized sufficient materials to enable root initials to grow, and to prolong indefinitely survival of the cuttings in water culture. Observations were not extended beyond six weeks, but during this period growth of leafy twigs and of roots continued to increase. Some of these observations may have practical horticultural applications in rooting woody shrubs and perhaps in prolonging the life of cut flowers.

Preliminary studies with related compounds such as vanillylacetone and isomeric hydroxynaphthal acetones suggest similar growth regulating possibilities. A series of related homologues and analogues is now under investigation. Some of these which seem to have potentialities as therapeutic agents for use in fungus infections of man will be reported later.

Summary

Salicylacetone, 4(*o*-hydroxyphenyl)-3-butenone-2, in concentrations of 1 mg./L. and above in the Sabouraud's agar, markedly inhibits growth of the dermatophyte *Trichophyton mentagrophytes*. Concentrations of 25 mg./L. in solutions tested by the agar cup technique cause pronounced inhibition. Salicylacetone also retards the growth of *Botrytis allii*, a fungus pathogen of plants.

Conversely, this compound, in concentrations from 304 to 200 mg./L. stimulates development of root initials, and their subsequent growth, from the buds of tubers of various varieties of potato (*Solanum tuberosum*) and on cuttings of *Ficus gnaptholocarpa*.

Dithiobiuret appears to be capable of stimulating development of leafy shoots on *Ficus gnaptholocarpa*. This compound was not tested on other plants. Combined with salicylacetone, it may act synergistically to promote development and growth of roots on cuttings. The latter effect is probably indirect, being due to increased shoot development which serves to provide organic material to support root growth.

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